In re Application of: SNYDER, R.O. et al.

Application No.:

10/798,192

Page 2 of 11

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

- Claim 1. (Currently Amended). A nucleic acid molecule comprising:
- (A) a first nucleotide sequence encoding an AAV Rep protein of a first serotype;
- (B) a second nucleotide sequence encoding an AAV Cap protein of a second serotype generated by amplifying of SEQ ID NO's.: 1-4; the second serotype being different from the first serotype; and
- (C) a third nucleotide sequence, encoding a transcription product having at least one containing an adenoviral sequence encoding a VA Adenoviral helper function, in reverse orientation to said first and second nucleotide sequences.
- Claim 2. (Original). The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is comprised within a vector.
- Claim 3. (Original). The nucleic acid molecule of claim 1, wherein the AAV Rep. protein is an AAV serotype 2 protein.
- Claim 4. (Original). The nucleic acid molecule of claim 1, wherein the AAV Rep protein is Rep52.
- Claim 5. (Original). The nucleic acid molecule of claim 1, wherein the AAV Rep protein is Rep78.
- Claim 6. (Original). The nucleic acid molecule of claim 4, wherein the first nucleotide sequence additionally encodes a Rep78 protein.

{WP285734,1}

T-884 P.06/14 F-030

In re Application of: SNYDER, R.O. et al.

Application No.:

10/798,192

Page 3 of 11

Claim 7. (Original). The nucleic acid molecule of claim 1, wherein the AAV Cap protein is an AAV serotype 1 Cap protein.

Claim 8. (Original). The nucleic acid molecule of claim 1, wherein the AAV Cap protein is an AAV serotype 5 Cap protein.

Claim 9. (Original). The nucleic acid molecule of claim 1, wherein the second nucleotide sequence encodes an AAV protein selected from the group consisting of: VP1, VP2, and VP3.

Claim 10. (Original). The nucleic acid molecule of claim 9, wherein the second nucleotide sequence encodes VP1, VP2, and VP3.

Claim 11. (Original). The nucleic acid molecule of claim 1, wherein the transcription product having at least one Adenoviral helper function is selected from the group consisting of: Adenovirus DNA binding protein, Adenovirus E4 protein, and Adenovirus virus associated RNA molecule.

Claim 12. (Original). The nucleic acid molecule of claim 2, wherein the nucleic acid is operably linked to at least one expression control sequence.

Claim 13. (Original). The nucleic acid molecule of claim 12, wherein the first nucleotide sequence encoding an AAV Rep protein of a first serotype is operably linked to a promoter.

Claim 14. (Original). The nucleic acid molecule of claim 13, wherein the promoter is selected from the group consisting of: AAV p5 and AAV p19 promoters.

(WP285734:1)

In re Application of: SNYDER, R.O. et al.

Application No.:

10/798,192

Page 4 of 11

Claim 15. (Original). The nucleic acid molecule of claim 12, wherein the second nucleotide sequence encoding an AAV Cap protein of a second serotype is operably linked to a promoter.

Claim 16. (Original). The nucleic acid molecule of claim 15, wherein the promoter is an AAV p40 promoter.

Claim 17. (Original). The nucleic acid molecule of claim 12, wherein the third nucleotide sequence encoding a transcription product having at least one Adenoviral helper function is operably linked to a promoter.

Claim 18. (Original). The nucleic acid molecule of claim 1, wherein the nucleic acid molecule further comprises a selectable marker.

Claim 19. (Original). The nucleic acid molecule of claim 18, wherein the selectable marker confers antibiotic resistance to a cell.

Claim 20. (Currently Amended). A cell comprising a nucleic acid molecule said nucleic acid molecule comprising:

- (A) a first nucleotide sequence encoding an AAV Rep protein of a first serotype;
- (B) a second nucleotide sequence encoding an AAV Cap protein of a second serotype generated by amplifying SEQ ID NO's.: 1-4; the second serotype being different from the first serotype; and,
- a third nucleotide sequence encoding a transcription product having-at (C) least one containing an adenoviral sequence encoding a VA Adenoviral helper function.
 - Claim 21. (Original). The cell of claim 20, wherein the cell is a mammalian cell.

{WP285734;1}

In an Amplication of

In re Application of: SNYDER, R.O. et al.

Application No.:

10/798,192

Page 5 of 11

Claim 22. (Original). The cell of claim 20, further comprising a second nucleic acid comprising a polynucleotide to be expressed interposed between a first AAV inverted terminal repeat and a second AAV inverted terminal repeat.

- Claim 23. (Original). The cell of claim 22, wherein the second nucleic acid is comprised within a vector.
- Claim 24. (Original). The cell of claim 23, wherein the first AAV inverted terminal repeat is an AAV serotype 2 inverted terminal repeat.
- Claim 25. (Original). The cell of claim 24, wherein the second AAV inverted terminal repeat is an AAV serotype 2 inverted terminal repeat.
- Claim 26. (Original). The cell of claim 22, wherein the polynucleotide encodes a protein.
- Claim 27. (Original). The cell of claim 22, wherein the polynucleotide encodes a selectable marker.
- Claim 28. (Original). The cell of claim 27, wherein the selectable marker is green fluorescent protein.
- Claim 29. (Currently Amended). A method of producing rAAV virions, the method comprising the steps of:
- (a) culturing a cell comprising a nucleic acid molecule comprising: a first nucleotide sequence encoding an AAV Rep protein of a first serotype; a second nucleotide sequence encoding an AAV Cap protein of a second serotype generated by amplifying SEQ ID NO's.: 1-4; the second serotype being different from the first serotype; and, a third nucleotide sequence encoding a transcription product having at least one containing an adenoviral sequence encoding a VA Adenoviral helper function whereby, rAAV virions are produced; and,

{WP285734,1}

T-884 P.09/14 F-030

In re Application of: SNYDER, R.O. et al.

Application No.:

10/798,192

Page 6 of 11

(b) isolating the rAAV virions produced from the cell.

Claim 30. (Original). The method of claim 29, wherein the cell is a mammalian cell.

Claim 31. (Previously presented). The method of claim 29, wherein the step (a) comprises culturing the cell into a culture medium.

Claim 32. (Original). The method of claim 31, wherein the step (b) of isolating the rAAV virious produced from the cell comprises separating the cell from the medium, lysing the cell to yield a cell lysate, and then isolating the rAAV virious from the cell lysate.

Claim 33. (Original). The method of claim 29, wherein the step (b) of isolating the rAAV virions produced from the cell comprises subjecting the produced rAAV virions to an iodixanol step gradient.

Claim 34. (Original). The method of claim 33, further comprising subjecting the produced rAAV virions to ion exchange chromatography.

Claim 35. (Original). The method of claim 34, wherein the produced rAAV virions contain at least one AAV serotype 1 capsid protein.

Claim 36. (Original). The method of claim 34, wherein the produced rAAV virions contain at least one AAV serotype 5 capsid protein.